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Heavy metals in the polychaete *Hediste diversicolor*, from Lake Burullus, Egypt

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ABSTRACT

Lake Burullus is a stressed wetland on the Egyptian Mediterranean coast, impacted by industrial, domestic and agricultural wastes. The polychaete *Hediste diversicolor* was used as a biomarker of some heavy metals in the lake, particularly Cd, Cu, Pb, and Zn. The levels of these metals were measured in the worms and surrounding sediment. The results indicated wide variation in the contents of heavy metals in the sediment and worms, with higher levels in the worms. The experimental work showed that *Hediste diversicolor* displayed a pronounced capacity to accumulate Cu and Pb, while the release of metals by the worms occurred at a lower rate. Lead and Copper showed similar mortality to the test-worms (51.7% and 51.6%, respectively) after 7 days at 500 ppm for each metal.

INTRODUCTION

Heavy metals are the most widespread and hazardous anthropogenic pollutants in the coastal marine waters (Martinez-Colon *et al*., 2009). They are usually concentrated in the sediment because of their rapid adsorption onto sediment particles (Dean, 2008), with harmful effects on biota (Mami *et al*., 2011).

Lake Burullus is one of the Egyptian lakes on the Mediterranean coast, lying under anthropogenic stress of huge amounts of waste waters from different sources (El-Zeiny and El-Kafrawy, 2017). Although the lake was registered in UNESCO as a RAMSAR site (Kassas, 2002; Khalil and El-Dawy, 2002), earlier studies reported that the lake received increasing quantities of waste waters without any pre-treatment (Okbah, 2005; Radwan, 2005; Zaghloul *et al*., 2007), causing the occurrence of widely different levels of heavy metals in its water and sediment, particularly during the past two decades (El-Nemr, 2003; Shaltout *et al*., 2005; Khalil *et al*., 2007; Chen *et al*., 2010; Hereher *et al*., 2010; Masoud *et al*., 2011; Eid *et al*., 2012; El-Baz, 2015; Nafea and Zyada, 2015; El-Amier *et al*., 2017).

Polychaetes play a great role in the health of benthic communities through sediment bioturbation (e.g. Poirier *et al.*, 2006; Watson *et al.*, 2007; Durou *et al.*, 2008; Rainbow *et* *al.,* 2009; Lewis and Watson, 2012). *Hediste diversicolor* is a surface-deposit feeder, herbivore, predator and a scavenger polychaete (Reise, 1979), commonly found in brackish waters and in the coastal areas (Diéz *et al*., 2000; Yilmaz, 2009). It is characterized by high physiological tolerance to extreme variation of many environmental parameters (Eriksen *et al*., 1988; Ait Alla *et al*., 2006; Bouraoui *et al.*, 2014). Due to its bioaccumulation capacity for heavy metals this worm was found to be able to inhabit metal-rich sediment (Rainbow, 1995; Dı´ez *et al*., 2000; Poirier *et al*., 2006), and could be used a bioindicator of environmental quality (Moreira *et al*., 2006; Poirier *et al*., 2006; Sun and Zhou, 2007; Durou *et al.*, 2008; Gillet *et al.*, 2008; Solé *et al*., 2009).

The aim of the present study was to investigate the levels of four heavy metals (Cd, Cu, Pb and Zn) in one of the abundant nereidid polychaete, *H. diversicolor,* in the sediment of Lake Burullus and the relationship between the concentrations of these metals inside the worm' body and in the surrounding sediment.

MATERIALS AND METHODS

The study area

Lake Burullus (Fig. 1) is the second largest lake on the Egyptian Mediterranean coast, experiencing anthropogenic stress (El-Kafrawy *et al*., 2015) of waste waters of different origin estimated by about 4.1 billion m^3/year (El-Amier *et al*., 2016) and 3.9 billion m^3/year (El-Zeiny and El-Kafrawy, 2017). The Lake occurs at 31^o 22' -31^o 26'N and 30^o 33'-31^o 07' E (Melegy *et al*., 2019), occupying an area of about 420 Km^2 with mainly muddy bottom and small percentage (19 %) of sand (Farag and El-Gamal, 2011). It has an average salinity of 3.51 ‰ (Shalaby *et al*., 2017), but it exposed to temporal variation in winter, particularly at the connection (Al-Bughaz) between the lake and the Mediterranean Sea (Emara *et al*., 2016).

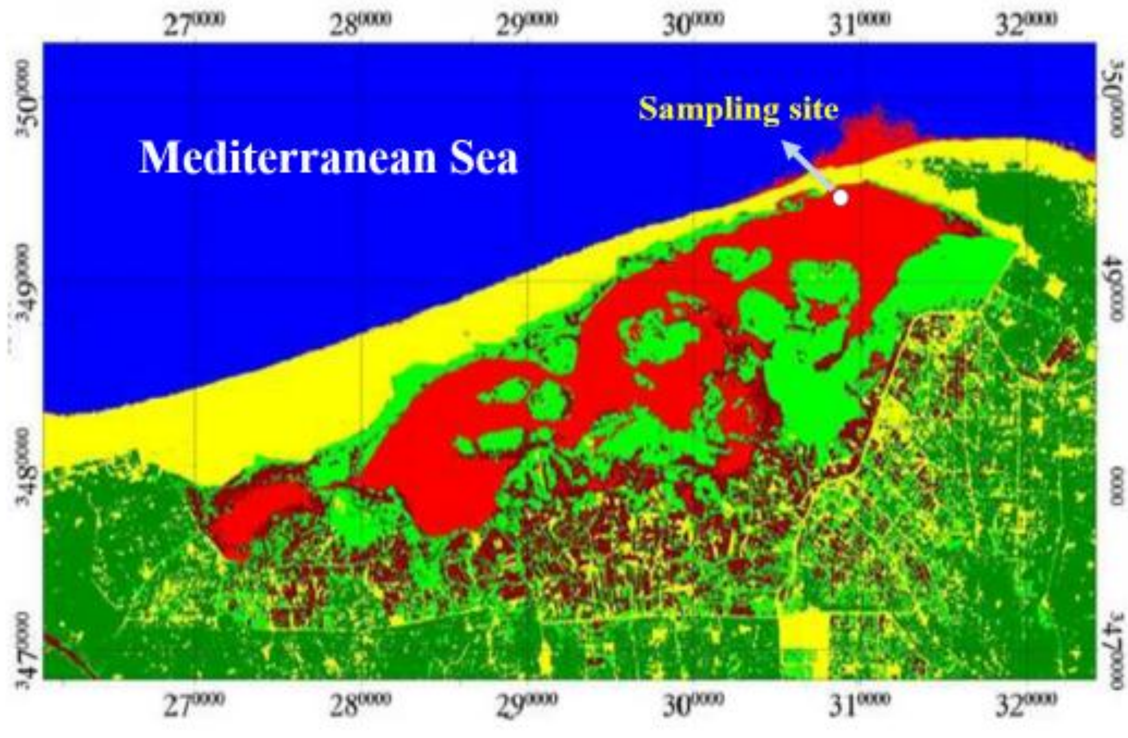


Figure 1. Lake Burullus and sampling site (After Google)

Sample collection

The *Hediste diversicolor* worms and sediment were collected frequently at a fixed site in Lake Burullus during the period from September 2018 to August 2019. The worms were collected from the upper 25 cm muddy sediment depth, placed in plastic bags filled with the lake water and kept cooled in ice box tell reaching the laboratory. The worms used for determination of heavy metals level were stored frozen at -20°C until analysis. For this analysis about ten worms of approximately similar size were dried at 60 °C for 24 h until fixed weight, ground in a mortar and a known dried weight was digested following Pini *et al*. (2015). For metal analysis in sediment, triplicate samples of 1 gm dried sediment were digested with a mixture of HCl, HNO_3 and HF according to the method described by Loring and Rantala (1990).

The heavy metals (Cd, Cu, Pb, and Zn) in both worms and sediment were measured using a computerized atomic absorption spectrometry (VARIAN, model AA800).

Biota-sediment accumulation factor (BSAF) was calculated following Bat *et al*. (2018).

Metals bioaccumulation

To assess the ability of the worm to accumulate heavy metals some experiments were carried out in the laboratory, using standard solutions of Lead (Pb) and copper (Cu). A certain concentration of each metal was placed in tri-replicates of 3 L glass round bottom aquaria, containing clean water with salinity (4 ppt) similar to that of Lake Burullus. In each aquarium, 30 worms of approximately similar size were introduced. Before starting the experiment, the level of each metal was measured in both the water and worms. The concentration of the metal was measured daily to determine the amount of the metal accumulated by the worms.

Depuration experiment

The efficiency of *H. diversicolor* to release the accumulated heavy metals was assessed, particularly for Pb and Cu. The experiment was carried out under laboratory conditions at temperature 20± 2°C and salinity 4‰ (under continuous aeration). After 4 h from collection, three replicates of 30 worms of approximately similar size were placed in 3 L glass aquaria containing 2 L of clean water. Before the experiment's start, the level of each metal was measured in the worms to define the amount of the accumulated metal in the worm during its existence in the Lake. After then, the metal content in the worm was measured in the three replicates at certain time intervals, and the depurated metal was calculated as the difference between each two successive periods of experimental time.

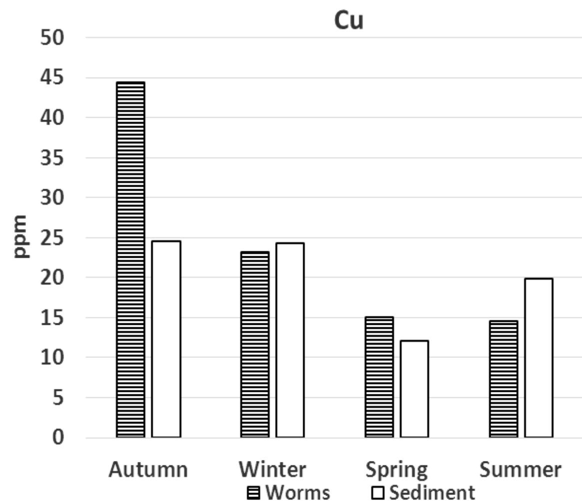
Mortality test

The sub-lethal dose of Pb and Cu to *H. diversicolor* was estimated under laboratory condition. Tri-replicates of 20 worms of approximately equal size were placed each in 3 L glass aquarium containing 2 L of clean water and certain amount of Pb or Cu. For Pb, the concentration used were 3000 ppm, 1000 ppm and 500 ppm, while for Cu, 2000, 1000, and 500 ppm were used. The number of died worms was counted every day to know the mortality percentage.

RESULTS

1.1. Heavy metals in sediment

The heavy metals concentrations in the sediment amounted to 20.17±9.87 ppm for Cu, 26.07 ±14.95 ppm for Pb, 34.66±15.99 ppm for Cd, and 45.48±20 ppm for Zn. The average values showed the following ranking: Zn> Cd > Pb> Cu. However, this pattern showed seasonal variation, being Zn> Cd > Pb> Cu in both summer and autumn, Zn> Pb> Cu> Cd in winter, and Cd> Zn> Pb> Cu in spring (Fig.2). Regarding the individual metal, Cu attained the highest level in autumn and winter, Pb in winter, Zn in summer, and Cd in spring. In contrast, Cu, Pb and Zn recorded the lowest levels during spring, while Cd was the lowest in winter (Fig.2).


Cu
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Worms
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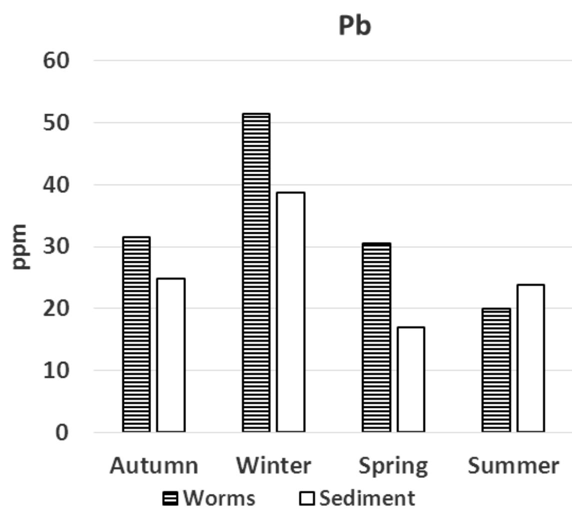
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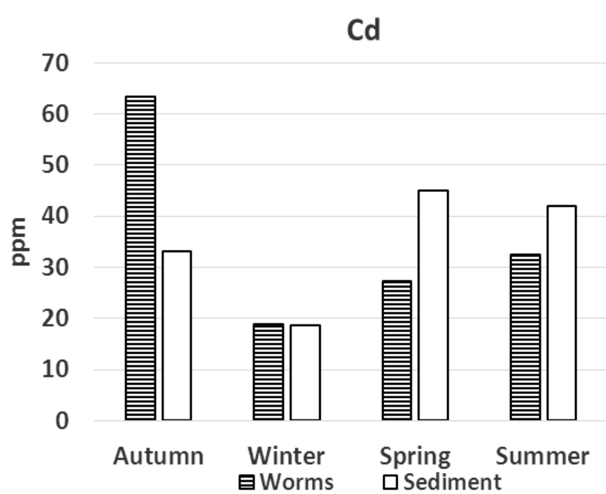
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Sediment

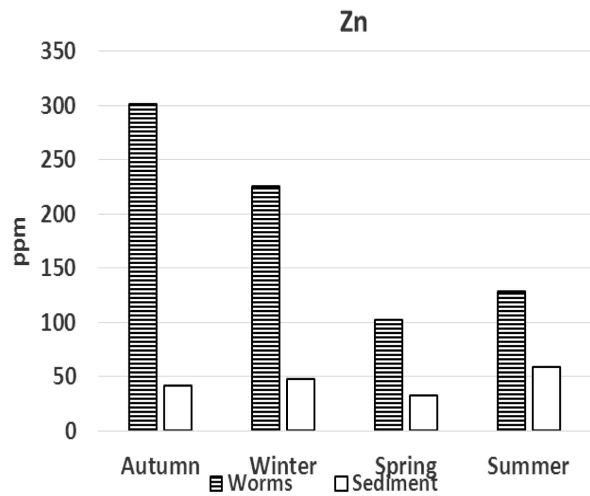

Figure 2. The seasonal variation of four heavy metals in *Hediste diversicolor* and sediment from Lake Burullus (September 2018 - August 2019)

1.2. Heavy metals in worms

The average level of the measured heavy metals in the whole soft tissues of *H. diversicolor* displayed similar ranking as in the sediment (Zn> Cd> Pb> Cu), but with different seasonal pattern. The trend was Zn> Cd> Cu> Pb in autumn, Zn> Pb> Cu> Cd in winter, Zn> Pb > Cd > Cu in spring and Zn> Cd> Pb > Cu in summer (Fig. 2). The highest level of Cd, Cu, and Zn was observed during autumn, while Pb attained its maximum level in winter. On the other hand, the lowest concentrations of Cu and Zn appeared during spring and summer, Pb in summer and Cd in winter (Fig. 2).

The worm-sediment concentration factor (CF) of the four measured metals displayed wide variability (Table 1). It varied between 0.4 - 3.0 for Cd, 0.6 - 3.2 for Cu, 0.7 - 2.9 for Pb, and 1.7 - 10.0 for Zn. The CF of Cu showed positive significant correlation with those of Cd and Zn (r = 0.7923 and 0.7778, respectively, n: 12, p: 0.05), while the values of both Cd and Zn was significantly correlated (r = 0.8596, n: 12, p: 0.05). As shown in Table 1, the CF was mostly >1, indicating that the worms accumulated higher heavy metals than the sediment. However, CF was sometimes < 1 which means the heavy metals are less in worm than in the sediment, particularly for Cu and Cd.

Table 1. The Monthly variation of worm-sediment concentration factor (CF) of the measured heavy metals**.**

	Cu	Pb	Cd	Zn
Sep. 2018	1.4	1.1	1.8	5.9
Oct.	3.2	1.1	3.0	10.0
Nov.	1.4	1.9	1.3	6.6
Dec.	1.6	1.6	1.0	7.6
Jan. 2019	0.6	1.6	1.0	4.2
Feb.	0.8	0.8	0.9	3.0
Mar.	1.9	1.9	0.7	2.5
Apr.	0.9	1.1	0.4	3.1
May.	1.0	2.9	0.9	3.5
Jun.	0.8	2.3	0.6	2.0
Jul.	0.6	0.7	0.5	1.7
Aug.	1.3	1.0	1.5	5.6

1.3. Metal accumulation

The experiment of Pb and Cu bioaccumulation by *H. diversicolor* showed that the two metals were accumulated by different percentages. As shown in Table (2), at 3000 ppb, the absorbed Pb increased gradually with time, whereas the concentration factor (CF) was 10.7 after the first day of exposure and rose to 17.1 after 3 days. For Cu, the worm displayed pronouncedly less bioaccumulation than Pb, as the CF of Cu at 1000 ppb was 7.1 after one day and increased to 11 after 5 days exposure (Table 2). *Hediste diversicolor* exhibited less efficiency toward Cd bioaccumulation, whereas the highest value of CF was observed after 2 days and amounted to 8.3 (Table 2). Meanwhile, in aquaria containing sediment and water the worm bioaccumulation of Pb appeared to be slightly higher than that occurred in the water only (Table 3).

Table 2. Metal accumulation by *Hediste diversicolor* in water **(**Av/: average concentration, BF: bioaccumulation factor)

Cd	3000ppm		Cu	1000ppb		Pb	3000 ppb	
	Av	CF		Av		Av	CF	CF
Initial conc	1.44		Initial conc	9.52		Initial conc.	35.59	
2 hrs	4.34	3.0	2 hrs	67.20	7.1	1 day	380.79	10.7
4 hrs	5.53	3.8	4 hrs	94.22	9.9	2 days	538.26	15.1
6 hrs	6.97	4.8	5 hrs	105.12	11.0	3 days	606.8	17.1
24 hrs	9.16	6.4						
48 hrs	11.91	8.3						

Table 3. Metal accumulation (ppm) by *Hediste diversicolor* in water and sediment**.**

	Water	**Sediment**	**Worms**	**Total**	**CF**
1 day	25.34	149.53	193.84	368.71	15.4
2 days	21.20	121.30	229.70	372.20	18.2
3 days	15.10	98.60	265.90	379.60	21.1
5 days	13.20	84.60	285.60	383.40	22.7

1.4. Depuration experiments

Hediste diversicolor demonstrated different percentages of depuration with the tested metals, as indicated from the depuration factor (DF). Table (4) revealed that after two days, the DF was 13.5 for Cd, 15.5 for Cu, 23.1 for Zn and 51.9 for Pb. These values displayed gradual increase for the all measured metals, reaching their maxima after 45 days. However, the maximum amount released by the worms appeared to be different for the four metals; the DF of Pb was the highest (94.4, followed by 88.4 for Zn, 88.1 for Cd, and the lowest value (53.2) for Cu (Table 4).

Table 4 Depuration percentages of heavy metals by *Hediste* diversicolor (DF: concentration of released metal/ Intial concentration of the metal).

		Initial Conc.	2 days	4 days	5 days	7 days	16 days	20 days	30 days	45 days
Cu	Conc. ppm	20.63	17.44	15.85	12.88	12.76	12.20	10.72	9.91	9.66
	DF		15.50	23.20	37.60	38.10	40.90	48.04	51.96	53.18
Pb	ppm	41.05	19.75	11.82	11.05	10.85	10.65	6.4902	3.39	2.32
	DF		51.90	71.21	73.08	73.57	74.06	84.19	91.74	94.35
Zn	ppm	648.43	498.60	384.45	341.577	127.93	118.64	97.98	95.67	75.30
	DF		23.10	40.70	47.32	80.27	81.70	84.89	85.24	88.39
Cd	ppm	1.85	1.60	1.42	1.02	0.85	0.63	0.43	0.32	0.22
	DF		13.50	23.24	44.86	54.05	65.94	76.76	82.70	88.10

1.5. Sublethal effect

The experiments of sublethal effect of Cu on *H. diversicolor* indicated that 2000 ppm caused LC100 after 3 hrs, 1000 ppm caused LC 100 after 8 hrs, while 500 ppm caused 65% mortality after 72 hrs (Table 5a).

Table 5a. The sublethal effect of Cu on *Hediste diversicolor* in water.

Cu	Death %	
2000 ppm	Contaminated	Control
2hr	38.3%	0%
3hr	100%	0%
1000 ppm	Contaminated	Control
4 hr	11.1%	0%
6hrs	56.6%	0%
8 hr	100%	0%
500 ppm	Contaminated	Control
1 day (24Hrs)	28.4%	0%
2 days (48 hrs)	41.7%	0%
3 days (72 hrs)	65 %	5 %

Lead showed less lethal effect on *H. diversicolor*, whereas 3000ppm caused the death of 55% of the tested worms after 3 days of exposure, 1000 ppm caused death of 58.5% after 4 days and 500ppm 51.6% after 6 days (Table 5b). On the other hand, when worms were placed in contaminated sediment containing 500 ppm Pb, no mortality was recorded during the first three days, while after 5 days the mortality was 26.5% only (Table 5c).

Table 5b. The sublethal effect of lead (Pb) on *H. diversicolor* in water**.**

Pb	Death %	
3000 ppm	Contaminated	Control
1 day	10	0
2 days	21.7	10
3 days	55.0	10
1000 ppm	Contaminated	Control
1 day	0	0
2 days	36.5	0
3 days	52.9	5
4 days	58.5	7
500ppm	Contaminated	Control
1 day	0%	0%
2 days	0 %	0 %
3 days	20 %	0 %
5 days	38.4 %	0 %
6 days	51.6 %	0 %

Table 5c. The sublethal effect of Lead on *H. diversicolor* in water and sediment.

Pb	Death %	
500 ppm	Contaminated	Control
1 day	0	0
2 days	0	0
3 days	0	0
5 days	26.5	0

DISCUSSION

The present study revealed that the measured heavy metals in the sediment of Lake Burullus were considerably different from those recorded previously. As shown in Table 6, Cd was pronouncedly higher than some of previously recorded values (Masoud *et al*., 2011; Nafea and Zyada, 2015; El-Amier *et al*., 2017; Shalaby *et al*., 2017), but it was close to the values observed by Saeed and Shaker (2008) and Shaltout *et al*. (2005). In contrast, the present level of Pb appeared to be lower than the values given by some authors (e.g. Shaltout *et al*., 2005; Saeed and Shaker, 2008*;* El-Amier *et al*., 2017), while it was higher than others (Chen *et al., 2010;* Masoud *et al*., 2011; Gu *et al*., 2013; Samy and El Bady, 2014*;* Shalaby *et al., 2017).* Both Cu and Zn appeared to be lower than those reported in most of the previous studies in Lake Burullus, with few exceptions (Table 6). The wide differences in the sediment contents of heavy metals were also observed in other world aquatic habitats (Table 7). Such differences are related to the quality and quantity of the discharged waste into each habitat (Chen *et al*., 2010; Hereher *et al*., 2010; Aydin-Onen *et al*., 2015; El-Baz, 2015; Nafea and Zyada, 2015; Emara *et al*., 2016). The high level of Pb in the sediment could be related to human activities such as fishing boat paintings, polluted wastes and discharges, municipal runoffs, and atmospheric deposition (Aydin-Onen *et al*., 2015).

Table 6. Historical records of the heavy metals (ppm) in sediment of Lake Burullus.

Cd	Cu	Pb	Zn	Reference
7.4 - 12.34	32.27 - 167.1	46.18 - 80.35	44.83 - 141.37	El Nemr (2003)
0.8 – 45.9	9.8 – 47.3	8.7 – 54.7	22.2 – 119.7	Shaltout *et al*. (2005)
ND - 41.49	27.13-77.02	ND - 81.32	72.77- 726.90	Saeed and Shaker (2008*)*
0.086	48.04	8.22	96.5	Chen *et al*.(2010)
5.84 – 16.42	50.3– 129.4	1.04 -9.33	163. – 482.8	Masoud *et al*. (2011)
1.48	34	15	121	Gu *et al.* (2013)
ND	50.19	9	83.25	Samy and El Bady (2014)
3.2 - 8.5	19.4–47.9	6.5 – 27.5	24.2 – 97.2	Nafea and Zyada (2015)
ND	0.18 – 6.02	ND	0.03 - 6.73	El Baz (2015)
8.24	59.81	17.85	94.33	Shalaby *et al*. (2017)
0.21 - 1.61	1.85 – 74.0	3.43-83.66	11.97 - 352.2	El-Amier *et al*. (2017)
34.66	20.17	26.07	45.48	Present study

Table 7. Heavy metals (ppm) in sediment in different areas

Area	**Cd**	**Cu**	**Pb**	**Zn**	Reference
Eastern Aegean	0.037–0.38	2.1-50.1	2.8 and 6.0	96.1 - 209.5	Aydin-Onen *et al*. (2015)
Spain	0±01–7.8±3.4	21±3.4–262±98	0.3±0.5	136±19–181± 26	Saiz-Salinaz and Francés-Zubillaga (1997)
Venetian lagoon (Italy)	0.25–17.8	NR	NR	177–358	Volpi Ghirardini *et al*. (1999)
lagoon of Venice (Italy)	1.33 ± 1.3	19.53 ± 20.66	40.89 ± 31.2	NR	Frangipane *et al*. (2005)
Seine estuary	1.13±0.05	48±7	NR	166 ±37	Berthet *et al*. (2003)
Restronguet Greek	(2.76±0.20	4413±262	NR	173	Berthet *et al*. (2003)
Boulogne harbor	0.38 ±0.01	153±10	NR	88	Berthet *et al*. (2003)
Bay of Somme	(0.05±0.00	0.64± 0.11	NR	105±3	Berthet *et al*. (2003)
Blackwater	0.19±0.01	53±16	NR	152	Berthet *et al*. (2003)
Moroccan lagoons	0.37±0.08	17±2	3.0±1.1	115±30	Idardare *et al*. (2008)
Homa lagoon	0.062±0.00	3.86±0.19, 17.2–41.0	12.54±0.39	58.90±1.83	Dora *et al*. (2007)
Tunisia	4.86- 37.42	13 - 98	18.98- 233.46	39.92- 574.89	Bahloul *et al*. (2018)

Although the measured metals in sediments of Lake Burullus ranked as follows: Zn > Cd > Pb> Cu, this rank experienced seasonal variation, particularly in winter (Zn> Pb> Cu> Cd), and spring (Cd > Zn > Pb > Cu). This pattern was different from those recorded previously in the Lake (EI-Sammak and El-Sabrouti, 1995; Shaltout *et al*., 2005; Saeed and Shaker, 2008; Masoud *et al*., 2011; El-Amier *et al*., 2017; El-Zeiny and El-Kafrawy, 2017) as well as in different aquatic habitats (Aydin-Onen *et al*., 2015).

Although no previous studies were done on heavy metals in *H. diversicolor* from Lake Burullus or any other Egyptian aquatic habitat, the available data demonstrated widely variable concentrations of heavy metals in this worm from different localities (Table 8). The Cd and Pb in the worms of Lake Burullus were pronouncedly higher than those found anywhere, while Cu was mostly lower except those recorded by Berthet *et al*. (2003) and Idardare *et al*. (2008). In contrast, Zn experienced considerably wide differences in the worm from different regions (Table 8).

Table 8. Heavy metals (ppm) in *H. diversicolor* in different areas.

Area	**Cd**	**Cu**	**Pb**	**Zn**	Ref.
Eastern Aegean	0.025 - 0.103	7.5 -30.6	2.8 - 6.0	96.1 - 209.5	Aydin-Onen *et al*. (2015)
Venetian lagoon	0.04–0.55	NR	NR	177–358	Volpi Ghirardini *et al*. (1999)
lagoon of Venice (Italy)	0.01 - 0.35	0.85 - 39.44	0.31 - 1.54	NR	Frangipane *et al*. (2005)
Estuary of Urdaibai	0.1–1.7	6.3–39	0–10	25–307	Díéz *et al*. (2000)
Blackwater	0.221	44	NR	152	Berthet *et al*. (2003)
Seine estuary	0.33±0.09	26±11	NR	166 ±37	Berthet *et al*. (2003)
Restronguet Creek	0.6 µg/g	3940	NR	173	Berthet *et al*. (2003)
Venice lagoon (Italy)	0.7–0.30	15.08–39.44	0.68–1.54	NR	Frangipane *et al*. (2005)
Ria Formosa lagoon and Cádiz Bay	0.04±0.01 - 1.8	2.66±0.65 - 37.47±11.88	0.30±0.05 - 6.84±1.50	26.13±4.65 - 257.50±37.41	Gomez *et al*. (2013)
Ria de Bilbao, Spain	not detectable (nd)–0.1±0.1	NR	0.3±0.5	136±19–181±26	Saiz Salinaz and Francés Zubillaga (1997)
Bay of Somme (	0.05±0.03	11±4	NR	105±3	Berthet *et al*. (2003)
Boulogne harbor	0.06	15	NR	88	Berthet *et al*. (2003)
Homa lagoon	0.01–0.16	1.54–10.10, 27.0±0.99–29.1±1.0	0.23–15.80, 11.27± 0.41–12.54±0.39	57.05±2.26–58.90±1.83	Dora *et al*. (2007), Taş *et al*. (2009)
Moroccan lagoons	0.09±0.6	8.8± 3.8	3.0±1.1	115±30	Idardare *et al*. (2008)
	326.35±323.1	784.98 ±878.58	191.31±138.85		Said *et al*. (2017)
	984.62±519	1261.2±706.05	191.31±138.85		Said *et al*. (2017)
Lake Burullus	35.46	24.22	33.34	189.64	Present study

The sequence of the four metals in *H. diversicolor* was approximately similar to that in sediment (Zn > Cd > Pb > Cu), but with different seasonal pattern. Aydin-Onen *et al*. (2015) observed that these metals were sequenced in *H. diversicolor* as follows Zn > Cu > Pb > Cd, respectively. The Biota-sediment accumulation factor (BSAF) is generally used to evaluate the efficiency of metal bioaccumulation in the tissues or organs of the organisms (Thomann *et al*. 1995) with value below or above 1 (Frangipane et al. 2005).

During the present study the BSAF mostly sustained values > 1 (1.1 – 1.5) for Cd, Cu and Pb, but it reached an average value of 4.64 for Zn. These differences can be explained by the ecological characteristics of the species (Aydin-Onen *et al*. 2015). A significant inverse correlation was observed between BSAF of some metals (Cd, Cu) and organic matter (OM) in sedimnt (Frangipane *et al*., 2005). This correlation was not detected in Lake Burullus during our study. On the other hand, in *H. diversicolor*, Cd showed the highest CF while Pb showed the lowest value (Bat *et al*. 2018), against the highest CF for Zn and the lowest for Cd in our study.

The variation of heavy metals in *H. diversicolor* could be explained by several factors, like their concentration in the surrounding sediment, the ability of the worm to absorb different metals, and to regulate their bioaccumulation (Bryan and Hummerstone, 1971). Diet or feeding habits may play a role in the variation of metal concentration in *H. diversicolor* (Lucas and Bertru, 1997).

According to Pini *et al*. (2015), a number of methods was developed to propose guidelines of metal concentrations in the sediment that pose potential adverse effects on aquatic life (Crane, 2003; Hübner *et al*. 2009; Luoma and Rainbow, 2008).

The permissible level in sediment was 6 ppm for Cd and 25 ppm for Cu (WHO, 2004), 10 ppm for Pb (USEPA, 1999), and 123 ppm for Zn (USEPA, 2002). In Lake Burullus, higher levels were frequently observed during the present study, indicating the continuous effect of the anthropogenic stress.

The relation between the metal levels in sediment and in polychaetes was subjected to wide scientific debate. Some studies recorded positive relationship in *H. diversicolor* (e.g. Bryan and Hummerstone, 1971; Zhou *et al*., 2004; Frangipane *et al*., 2005; Rainbow *et al*., 2009), while others observed weak relationship or even absent (e.g. Otero *et al*., 2000; Berthet *et al*., 2003; Poirier *et al.*, 2006; Amiard *et al*., 2007; Durou *et al*. 2007; Idardare *et al*., 2008; Aydin-Onen *et al*., 2015).

Such discrepancies may be attributed to the complex interactions between metal bioavailability and physiological factors which control the metal bioaccumulation (Frangipane *et al*., 2005), and the animal's reproductive cycle (Idardare *et al*., 2008). On the other hand, the uptake of metals may be regulated by the balance between uptake and excretion rates (Luoma and Rainbow, 2008), or by detoxification processes (Mouneyrac *et al*., 2003; Greim and Snyder, 2008; Casado-Martinez *et al.*, 2010).

The increase in metal concentration in the sediment was not always followed by additional metal bioaccumulation in *N. diversicolor* (Saiz-Salinas and Frances-Zubillaga, 1997; Berthet *et al*., 2003). For example, the metal concentrations were higher in the worms than in the sediment (Idardare *et al*., 2008; Aydin-Onen *et al*., 2015). This was also observed during the present study for the four measured metals.

By contrary, Pb in *H. diversicolor* was lower than in the sediments (Alam *et al*., 2010; Aydin-Onen *et al*., 2015; Bat *et al*., 2018), and this was attributed to the ability of the worm to secret mucus which reduces metal availability for uptake (Berthet *et al*., 2003; Mouneyrac *et al*., 2003; Zhou *et al*., 2004).

A positive significant correlation was found between Cu and Zn in the sediment of Lake Burullus. According to Aydin-Onen *et al*. (2015) such correlation between the two metals in sediments indicates their similar sources. (Frangipane *et al*., 2005) observed a significant correlation between Pb and Cu in sediments, but this correlation was less significant in Lake Burullus (r= 0.569 at $p<0.05$, n =12). In *H. diversicolor* from Lake Burullus, Cu was positively correlated with both Cd and Zn, while Cd was correlated with Zn at $p<0.1$. This agrees partially with Aydin-Onen *et al*. (2015) who reported a positive relationship between Cd and Cu in the same worm. On the other hand, Cd in *H. diversicolor* was significantly correlated with Zn in sediment (Aydin-Onen *et al*., 2015), but in the present study we recorded significant correlation between Pb in the sediment and in the worm. *Hediste diversicolor* has an ability to accumulate both Cu and Cd proportionally to their concentrations in the surrounding medium (Díez *et al*., 2000; Berthet *et al*., 2003).

The present study showed that Zn was the most enriched metal in Lake Burullus, followed by Cd, Pb, while Cu was the least metal in the Lake. Cadmium was reported as the most enriched element in the lake sediments, due to industrial and agricultural wastes (El-Amier *et al*., 2017). On seasonal bases, the Cd, Cu and Zn sustained the highest values during summer and high values during winter, except Pb which attained its maximum in summer and less so in spring. (El-Amier *et al*. (2017) observed the highest values of Cu and Zn in June, and Cd and Pb in August, but Eid *et al*. (2012) found the highest value of Pb in June, Cd and Cu in August, and Zn in February. Emara *et al*. (2016) recorded elevation of Cu in Lake Burullus during winter and spring, mainly due to the drainage water. In addition, Cu in Lake Burullus is released into water from painting of hundreds of fishing boats in the Lake (Hereher *et al*., 2010; El-Baz, 2015). Zinc significantly increased in agricultural wastewater in summer and autumn, with some elevations in Lake Burullus in spring, relative to the pollution of water with some industrial effluents (Hereher *et al*., 2010; El-Baz, 2015), particularly the discharged wastes of many textile companies from El-Mehalla El-Kobra (El-Baz, 2015), while Pb brought to the lake through the sewage discharge from the nearby cities of Tanta and Kafr El-Sheikh (Chen *et al*., 2010; Nafea and Zyada, 2015).

During the present study, *H. diversicolor* contained the highest levels of Cd, Cu, and Zn in autumn, and Pb in winter. Aydin-Onen *et al*. (2015) measured the highest Cd and Cu in September, while the maximum Pb and Zn values occurred in May. Such variations were attributed to the biological cycle (Cheggour *et al*., 1990), reproductive cycle and variations in body weight (Idardare *et al*., 2008), as the highest metal concentrations in the worm were found during periods of high gametogenic activity in autumn (Díez *et al*., 2000; Idardare *et al*., 2008; Aydin-Onen *et al*., 2015).

Salinity may also effect the bioavailabilty of metals in the *H. diversicolor* (e.g. Phelps *et al*., 1985), because low salinity increases the cupric ion activity (Zamuda and Saunda, 1982), causing the increase of the bioavailability of Cu to the ragworm (Ozoh, 1994). However, *H. diversicolor can* regulate the uptake of Cu, reflecting the prevailing biotic and abiotic conditions (Howard and Brown, 1983). On the other hand, increasing temperatures enhances the accumulation of heavy metals in the worm, especially Cu (Ozoh, 1994). This supports our findings in Lake Burullus, where all measured metals attained the maximum concentration in *H. diversicolor* during warm period.

The concentrations of Cu, Pb and Cd in *H. diversicolor* correlated strongly with their amounts in sediments (Bryan, 1974), indicting the ability of this worm to remove metals from the sediments (Bat and Raffaelli, 1998). But it was supposed that concentrations of metal in organisms usually show individual variability (Poirier *et al*., 2006), as some worms of similar weight and from the same site had widely different concentrations for Cd and Cu (Pini *et al*., 2015). Meanwhile, the amounts of accumulated metals in *H. diversicolor* appeared to be different relative to the size differences (Bat *et al*., 2018).

The depuration experiment indicated that after 45 days *H. diversicolor* released about 94% of Pb, 88.4 % of Zn, 88.1% of Cd, while 53.2% only of Cu was released. The low percentage of Cu release was reported by (Zhou *et al*., 2004; Geffard *et al*., 2005), while Howard and Brown (1983) mentioned that the worm lost approximately half the accumulated Cu after a certain period of exposure). This variation of Cu concentration in *H. diversicolor* is attributed to its starvation (Neuhoff, 1979). On the other hand, Howard and Brown (1983) reported that Zn absorbed by *H. diversicolor* is not lost in the short term, and this support our finding that Zn was released after 45 days. However, individual worms neither accumulated nor depurated at the same rate (Ozoh, 1994).

The present study recorded 51.7% lethal in *H. diversicolor* at 500 ppm Pb and 51.6% 500 ppm Cu after 144 hrs. Moreira *et al*. (2005) recorded LC50 for post exposure feeding *H. diversicolor* at Cu 241 and 125 ppm after 48-hrs and 96-hrs respectively.

CONCLUSION

The resent study revealed that the polychaete *Hediste diversicolor* in Lake Burullus contained higher concentrations of heavy metals (Cd, Cu, Pb, and Zn) than in the surrounding sediment, with wide temporal variation in the values of these metals in both sediments and worms. This study indicated also that *Hediste diversicolor* had high capacity to accumulate Cu and Pb, but it displayed low rate of releasing the accumulated metals.

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